(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 June 2004 (17.06.2004)

PCT

(10) International Publication Number
WO 2004/050687 A2

(51) International Patent Classification[7]: C07K 7/00

(21) International Application Number:
PCT/US2003/038627

(22) International Filing Date: 4 December 2003 (04.12.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/309,934 4 December 2002 (04.12.2002) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

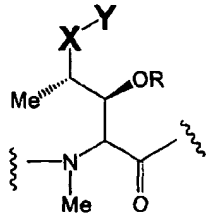
Published:

— *without international search report and to be republished upon receipt of that report*

[Continued on next page]

(54) Title: CYCLOSPORINS FOR THE TREATMENT OF AUTOIMMUNE DISEASES

A---B---Sar-MeLeu-Val-MeLeu-Ala---U---MeLeu-MeLeu-MeVal
1 2 3 8 11 (I)



(II)

(A)

(57) Abstract: The present invention provides a cyclosporin of the following Formula (I), Formula (I) or a pharmaceutically acceptable salt, ester or prodrug thereof. In Formula (I), A is Formula (II) wherein: X is selected from the group consisting of: -(CH2)$_n$ and -CH2-CH=CH-(CH$_2$)$_m$-, where n is an integer of from 1 to 8 and m is an integer of from 2 to 5; Y is selected form the group consisting of: OH, OAc, halogen, N_3, CN and OS(O)$_2$R$_{10}$, wherein R$_{10}$ is selected from the group consisting of F, CH_3, CF_3, Ph, MePh; or, alternatively, X and Y taken together are selected from the group consisting of: -CH=CH$_2$, -CHO, and -CH$_2$CH$_3$; R is selected from the group consisting of: hydrogen and a hydroxyl protecting group; B is selected from the group consisting of: -αAbu-, -Val-, -Thr- and -Nva-; and U is selected from the group consisting of: -(D)Ala-, -(D)Ser-, -[O-(2 hydroxyethyl)(D)Ser]-, -[O-acyl(D)Ser]- and -[O-(2-acyloxyethyl)(D)Ser]-.

WO 2004/050687 A2

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

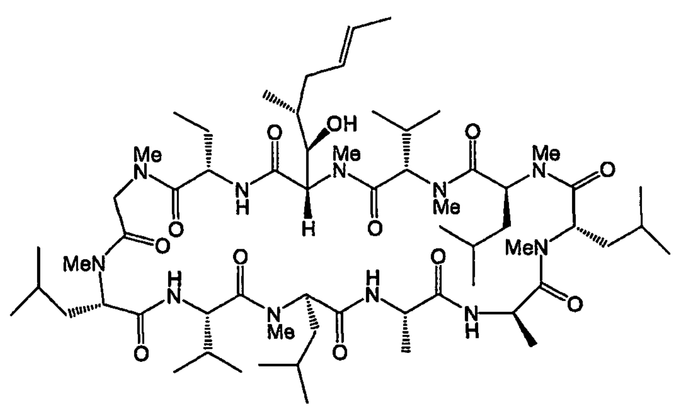
CYCLOSPORINS FOR THE TREATMENT OF IMMUNE DISORDERS

Technical Field

The present invention relates to novel semisynthetic cyclosporin analogs for the prevention of organ transplantation rejection and the treatment of immune disorders and inflammation, their use as pharmaceuticals and pharmaceutical compositions comprising them, as well as the processes for their production.

Background of the Invention

The cyclosporins comprise a class of structurally distinctive, cyclic, poly-N-methylated undecapeptides, commonly possessing pharmacological, in particular immunosuppressive, anti-inflammatory and antiparasitic activity. The first of the cyclosporins to be isolated was the naturally occurring fungal metabolite Ciclosporin or Cyclosporin, also known as cyclosporin A.



Cyclosporin A

MeBmt-αAbu-Sar-MeLeu-Val-MeLeu-Ala-DAla-MeLeu-MeLeu-MeVal
1 2 3 8 11

Since the original discovery of Cyclosporin, a wide variety of naturally occurring cyclosporins have been isolated and identified, and many further non-natural cyclosporins have been prepared by total- or semi-synthetic means or by the application of modified culture techniques. The class comprised by the

cyclosporins is thus now substantial and includes, for example, the naturally occurring cyclosporins A through Z [cf., Traber et al.;1, Helv. Chim. Acta, 60, 1247-1255 (1977); Traber et al.; 2, Helv. Chim. Acta, 65, 1655-1667 (1982); Kobel et al.; Europ. J. Applied Microbiology and Biotechnology, 14, 273-240 (1982); and von Wartburg et al.; Progress in Allergy, 38, 28-45 (1986)], as well as various non-natural cyclosporin derivatives and artificial or synthetic cyclosporin derivatives and artificial or synthetic cyclosporins including dihydrocyclosporins [in which the the –MeBmt-residue is saturated by hydrogenation]; derivatized cyclosporins (e.g., in which the 3'-O-atom of the –MeBmt- residue is acylated or a further substituent is introduced at the α-carbon atom of the sarcosyl residue at the 3-position); and cyclosporins in which variant amino acids are incorporated at specific positions within the peptide sequence, e.g. employing the total synthetic method for the production of cyclosporins developed by R. Wenger—see e.g. Traber et al., 1; Traber et al., 2; and Kobel et al., loc cit. U.S. Pat. Nos. 4,108,985, 4,220,641, 4,288,431, 4,554,351, 4,396,542 and 4,798,823; European Patent Publication Nos. 34,567A, 56,782A, 300,784A and 300,785; International Patent Publication No. WO 86/02080 and UK Patent Publication Nos. 2,206,119 and 2,207,678; Wenger 1, Transpl. Proc., 15 Suppl. 1:2230 (1983); Wenger 2, Angew. Chem. Int. Ed. 24 77 (1985) and Wenger 3, Progress in the Chemistry of Organic Natural Products, 50, 123 (1986).

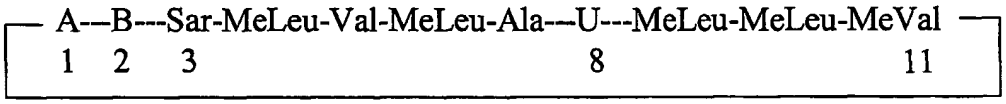
The compound cyclosporine (cyclosporine A or CsA) has found wide use since its introduction in the fields of organ transplantation and immunomodulation, and has brought about a significant increase in the success rate for transplantation procedures. Undesired side effects associated with cyclosporine, however, such as nephrotoxicity, have led to a continued search for immunosuppressant compounds having improved, efficacy and safety.

Side effects with systemic CsA include increase in diastolic blood pressure and decrease in renal function. Other side effects include hepatic dysfunction, hypertrichosis, tremor, gingival hyperplasis and paraesthsia. The systemic toxicity of CsA limits its use for the treatment of certain diseases.

Summary of the Invention

The present invention relates to novel cyclosporin analogs and methods of treatment for the prevention of organ transplantation rejection and the treatment of immune disorders or inflammation in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production.

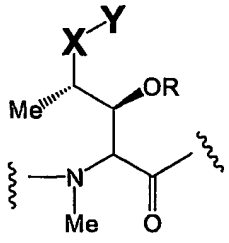
More particularly, the present invention provides a cyclosporin of the following Formula (I),



(I)

or a pharmaceutically acceptable salt, ester or prodrug thereof.

In Formula (I), A is



(A),

wherein:

X is selected from the group consisting of: $-(CH_2)_n-$ and $-CH_2-CH=CH-(CH_2)_m-$, where n is an integer of from 1 to 8 and m is an integer of from 2 to 5;

Y is selected form the group consisting of: OH, OAc, halogen, N_3, CN and $OS(O)_2R_{10}$, wherein R_{10} is selected from the group consisting of F, CH_3, CF_3, Ph, MePh;

or, alternatively, X and Y taken together are selected from the group consisting of: $-CH=CH_2$, –CHO, and $-CH_2CH_3$;

R is selected from the group consisting of: hydrogen and a hydroxyl protecting group;

B is selected from the group consisting of: –αAbu-, -Val-, -Thr- and –Nva-; and

U is selected from the group consisting of: –(D)Ala-, -(D)Ser-, –[O-(2 hydroxyethyl)(D)Ser]-, -[O-acyl(D)Ser]- and –[O-(2-acyloxyethyl)(D)Ser]-.

In Formula (I), amino acid residues referred to by abbreviation, eg. –Ala-, -MeLeu-, -αAbu-, etc., are, in accordance with conventional practice, to be understood as having the L-configuration unless otherwise indicated. (For example, –(D)Ala- represents a residue having the D-configuration). Residue abbreviation preceeded by "Me" as in the case of "MeLeu", represents an α-N-methylated residue. Individual residues of the cyclosporin molecule are numbered, as in the art, clockwise and starting with the residue, -MeBmt- corresponding to residue 1. The same numerical sequence is employed throughout the present specifications and claims.

Preferred cyclosporin analogs of the present invention are compounds represented by Formula I, where X is $–(CH_2)_2–$, Y is N_3, B is –αAbu– and U is –(D)Ala–, and (A) and R are as previously defined, or a pharmaceutically acceptable salt, ester or prodrug thereof.

Accordingly, the present invention provides the use of cyclosporin analogs for the manufacture of a preparation for the treatment, with or without the concurrent use of other drugs, of organ transplantation rejections, immune disorders, and inflammation including rheumatoid arithis, psoriasis, inflammatory bowel diseases, COPD, allergic rhinitis, and asthma.

Detailed Description of the Invention

A first embodiment of the invention is a compound represented by Formula I as described above, or a pharmaceutically acceptable salt, ester or prodrug thereof.

Representative compounds of the invention include, but are not limited to, the compounds selected from the group consisting of:

Compound of Formula (I): (A), X = $-(CH_2)_2-$, Y = OH, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = $OS(O)_2CH_3$, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = N_3, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = N_3, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = CN, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = CN, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = F, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = F, R = H, B = –αAbu- and U = –(D)Ala-,

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = Cl, R = Ac, B = –αAbu- and U = –(D)Ala-,

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = Cl, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = Br, R = H, B = –αAbu- and U = –(D)Ala-,

Compound of Formula (I): (A): X andY taken together = -CH=CH_2, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X andY taken together = -CH=CH_2, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CH_2CH_3, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CHO, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CHO, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CH_2OH, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -$CH_2OS(O)_2CH_3$, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CH_2N_3, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CH_2N_3, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CH_2CN, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CH_2F, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CH_2Cl, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CH=CH-$(CH_2)_3$-OAc, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = CH_2-CH=CH-$(CH_2)_3N_3$, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = CH_2-CH=CH-$(CH_2)_2$-OAc, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = CH_2-CH=CH-$(CH_2)_4$-OAc, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = CH_2-CH=CH-$(CH_2)_3$-Br, R = H, B = –αAbu- and U = –(D)Ala-; and

Compound of Formula (I): (A): X and Y taken together = CH_2-CH=CH-$(CH_2)_3$-CN, R = H, B = –αAbu- and U = –(D)Ala-.

The potent immunomodulatory activity which compounds of the instant invention demonstrate in common *in vitro* biological assays (for example, calcineurin phosphatase and binding assays, NFAT reporter gene assay, murine and human mixed lymphocyte reaction) or animal models (for example delayed-type hypersensitivity response - DTH,–allergan induced pulmonary eosinophilia) indicate that these compounds possess immunosuppressive, antimicrobial, antifungal, antiviral, antiinflammatory, and antiproliferative activity, and possess the ability to reverse chemotherapeutic drug resistance. As agents block T-cell activation, a prerequisite for HIV proliferation, the compounds are useful as prophylactics for the prevention of HIV replication. The compounds of the invention would be useful when used alone, or in combination therapy with other immunosuppressants, for example, but not limited to, FK506, rapamycin,

cyclosporin A, picibanil, mycophenolic acid, azathioprine, prednisolone, cyclophosphamide, brequinar and leflunomide.

As immunosuppressants, the compounds of the present invention are useful when administered for the prevention of immune-mediated tissue or organ graft rejection. Examples of transplanted tissues and organs which suffer from these effects are heart, kidney, liver, medulla ossium, skin, cornea, lung, pancreas, intestinum tenue, limb, muscle, nervus, duodenum, small-bowel, pancreatic-islet-cell, and the like; as well as graft-versus-host diseases brought about by medulla ossium transplantation. The regulation of the immune response by the compounds of the invention would also find utility in the treatment of autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosis, hyperimmunoglobulin E, Hashimoto's thyroiditis, multiple sclerosis, progressive systemic sclerosis, myasthenia gravis, type I diabetes, uveitis, allergic encephalomyelitis, glomerulonephritis, and the like; and further infectious diseases caused by pathogenic microorganisms, such as HIV. In the particular cases of HIV-1, HIV-2 and related retroviral strains, inhibition of T-cell mitosis would suppress the replication of the virus, since the virus relies upon the host T-cell's proliferative functions to replicate.

Further uses include the treatment and prophylaxis of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses, such as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise) such as keratoconjunctivitis, vernal conjunctivitis, keratitis, herpetic keratitis, conical cornea, dystrophia epithelialis corneae, corneal leukoma, ocular pemphigus, Mooren's ulcer, Scleritis, Graves' opthalmopathy, Vogt-Koyanagi-Harada syndrome, sarcoidosis, multiple myeloma, etc.; obstructive airway diseases, which includes conditions such as COPD, asthma (for example, bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma), particularly chronic or inveterate asthma (for example, late asthma and airway hyper-responsiveness),

bronchitis, allergic rhinitis and the like; inflammation of mucosa and blood vessels such as gastric ulcers, vascular damage caused by ischemic diseases and thrombosis. Moreover, hyperproliferative vascular diseases such as intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion, particularly following biologically- or mechanically-mediated vascular injury can be treated or prevented by the compounds of the invention.

Other treatable conditions would include but are not limited to ischemic bowel diseases, inflammatory bowel diseases, necrotizing enterocolitis, intestinal lesions associated with thermal burns and leukotriene B_4-mediated diseases; intestinal inflammations/allergies such as Coeliac diseases, proctitis, eosinophilic gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis; food-related allergic diseases which have symptomatic manifestation remote from the gastro-intestinal tract (e.g., migraine, rhinitis and eczema); renal diseases such as interstitial nephritis, Goodpasture's syndrome, hemolytic-uremic syndrome and diabetic nephropathy; nervous diseases such as multiple myositis, Guillain-Barre syndrome, Meniere's disease, polyneuritis, multiple neuritis, mononeuritis and radiculopathy; endocrine diseases such as hyperthyroidism and Basedow's disease; hematic diseases such as pure red cell aplasia, aplastic anemia, hypoplastic anemia, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, agranulocytosis, pernicious anemia, megaloblastic anemia and anerythroplasia; bone diseases such as osteoporosis; respiratory diseases such as sarcoidosis, fibroid lung and idiopathic interstitial pneumonia; skin disease such as dermatomyositis, leukoderma vulgaris, ichthyosis vulgaris, photoallergic sensitivity and cutaneous T cell lymphoma; circulatory diseases such as arteriosclerosis, atherosclerosis, aortitis syndrome, polyarteritis nodosa and myocardosis; collagen diseases such as scleroderma, Wegener's granuloma and Sjogren's syndrome; adiposis; eosinophilic fasciitis; periodontal disease such as lesions of gingiva, periodontium, alveolar bone and substantia ossea dentis; nephrotic syndrome such as glomerulonephritis; male pattern aleopecia or alopecia senilis by preventing epilation or providing hair germination and/or promoting hair generation and hair growth; muscular dystrophy; Pyoderma and Sezary's syndrome; Addison's disease; active oxygen-mediated diseases, as for example organ injury such as ischemia-reperfusion injury of organs (such as heart, liver, kidney and digestive

tract) which occurs upon preservation, transplantation or ischemic disease (for example, thrombosis and cardiac infraction): intestinal diseases such as endotoxin-shock, pseudomembranous colitis and colitis caused by drug or radiation; renal diseases such as ischemic acute renal insufficiency and chronic renal insufficiency; pulmonary diseases such as toxinosis caused by lung-oxygen or drug (for example, paracort and bleomycins), lung cancer and pulmonary emphysema; ocular diseases such as cataracta, siderosis, retinitis, pigmentosa, senile macular degeneration, vitreal scarring and corneal alkali burn; dermatitis such as erythema multiforme, linear IgA ballous dermatitis and cement dermatitis; and others such as gingivitis, periodontitis, sepsis, pancreatitis, diseases caused by environmental pollution (for example, air pollution), aging, carcinogenis, metastasis of carcinoma and hypobaropathy; disease caused by histamine or leukotriene-C_4 release; Behcet's disease such as intestinal-, vasculo- or neuro-Behcet's disease, and also Behcet's which affects the oral cavity, skin, eye, vulva, articulation, epididymis, lung, kidney and so on. Furthermore, the compounds of the invention are useful for the treatment and prevention of hepatic disease such as immunogenic diseases (for example, chronic autoimmune liver diseases such as the group consisting of autoimmune hepatitis, primary biliary cirrhosis and sclerosing cholangitis), partial liver resection, acute liver necrosis (e.g., necrosis caused by toxin, viral hepatitis, shock or anoxia), B-virus hepatitis, non-A/non-B hepatitis, cirrhosis (such as alcoholic cirrhosis) and hepatic failure such as fulminant hepatic failure, late-onset hepatic failure and "acute-on-chronic" liver failure (acute liver failure on chronic liver diseases), and moreover are useful for various diseases because of their useful activity such as augmention of chemotherapeutic effect, preventing or treating activity of cytomegalovirus infection, particularly HCMV infection, anti-inflammatory activity, and so on.

The compounds of the present invention may be used as vaccines to treat immunosuppression in a subject. It is sometimes found that the antigen introduced into the body for the acquisition of immunity from disease also acts as an immunosuppressive agent, and therefore, antibodies are not produced by the body and immunity is not acquired. By introducing a compound of the present invention into the body as a vaccine, the undesired immunosuppression may be overcome and immunity acquired.

The compounds of the present invention may also find utility in the chemosensitization of drug resistant target cells. Cyclosporin A and FK-506 are known to be effective modulators of P-glycoprotein, a substance which binds to and inhibits the action of anticancer drugs by inhibiting P-glycoprotein, as they are capable of increasing the sensitivity of multidrug resistant (MDR) cells to chemotherapeutic agents. It is believed that the compounds of the invention may likewise be effective at overcoming resistance expressed to clinically useful antitumour drugs such as 5-fluorouracil, cisplatin, methotrexate, vincristine, vinblastine and adriamycin, colchicine and vincristine.

Further, it has recently been shown that the steroid receptor-associated heat shock proteins, hsp56 or hsp59, belong to the class of immunophilin proteins (*see* "HSP70 induction by cyclosporin A in cultured rat hepatocytes: effect of vitamin E succinate," Andres, David et al., *Instituto de Bioqimica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain*. J. Hepatol. (2000) 33(4), 570-579; "Cyclosporin A Induces an Atypical Heat Shock Response," Paslaru, Liliana, *et al.*, Unite de Genetique Moleculaire, Paris, Fr. Biochem. Biophys. Res. Commun. (2000), 269(2), 464-469; "The cyclosporine A –binding immunophilin CyP-40 and the FK506-binding immunophilin hsp56 bind to a common site on hsp90 and exist in independent cytosolic heterocomplexes with the untransformed glucocorticoid receptor," Owens-Grillo, Janet K. et al., Med. Sch., Univ. Michigan, Ann Arbor, MI USA. J. Biol. Chem. (1995), 270(35), 20479-84). The ability of a steroid receptor-associated heat shock protein to bind the immunosuppressive CsA suggests that the steroid receptor and immunophilin signal transduction pathways are functionally interrelated. The combined treatment of compounds of the present invention and low concentrations of a steroid ligand (for e.g., progesterone, dexamethasone) result in a significant enhancement of target gene expression over that seen in response to ligand alone. Thus, the compounds of the present invention potentiate steroid-mediated transactivation.

Aqueous liquid compositions of the present invention may be particularly useful for the treatment and prevention of various diseases of the eye such as autoimmune diseases (including, for example, conical cornea, keratitis, dysophia

epithelialis corneae, leukoma, Mooren's ulcer, sclevitis and Graves' ophthalmopathy) and rejection of corneal transplantation.

Accordingly, the pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a cyclosporin analog of the invention in combination with a pharmaceutically acceptable carrier or excipient. In particular, compositions pertaining to the present invention are useful for treating a subject for immune-mediated organ or tissue allograft rejection, a graft-versus-host disease, an autoimmune disease, an obstructive airway disease, a hyperproliferative disease, or an ischemic or inflammatory intestinal or bowel disease.

The present invention also relates to method(s) of treatment of immune disorders and inflammation or prevention of organ transplant rejection in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable excipients, as described throughout the present specification.

The methods of the present invention comprise treating a subject in need of immunosuppresive, anti-inflammatory, antimicrobial, antifungal, antiviral or antiproliferative therapy, or requiring the reversal of chemotherapeutic drug resistance, by administering a therapeutically effective amount of a compound of the invention for such time and in such amounts as is necessary to produce the desired result.

As used in the present invention, "therapeutically effective amount" of one of the compounds means a sufficient amount of the compound to treat a particular disease, at a reasonable benefit/ risk ratio. The compounds of the present invention may be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug forms. Alternatively, the compounds may be administered as pharmaceutical compositions containing the compound of interest in combination with one or more drugs or pharmaceutically acceptable excipients. It will be understood, however, that the total daily usage of

the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment.

The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

The total daily dose of the compounds of this invention administered to a human or lower animal may range from about 0.001 to about 10 mg/kg of a patient's body mass/day. For purposes of oral administration, more preferable doses may be in the range of from about 0.005 to about 3 mg/kg/day. If desired, the effective daily dose may be divided into multiple doses for purposes of administration; consequently, single dose compositions may contain such amounts or submultiples thereof to make up the daily dose.

Definitions

The term "hydroxy protecting group," as used herein, refers to an easily removable group which is known in the art to protect a hydroxyl group against undesirable reaction during synthetic procedures and to be selectively removable. The use of hydroxy-protecting groups is well known in the art for protecting groups against undesirable reactions during a synthetic procedure and many such protecting groups are known, see T.H. Greene and P.G. M. Wuts, Protective Groups in Organic Synthesis, 3rd edition, John Wiley & Sons, New York (1999), which is hereby incorporated by reference in its entirety. Examples of hydroxy protecting groups include, but are not limited to, acetyl, methylthiomethyl, *tert-*

dimethylsilyl, *tert*-butyldiphenylsilyl, acyl substituted with an aromatic group and the like.

The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977), which is incorporated herein by reference. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

As used herein, the term "pharmaceutically acceptable ester" refers to esters which hydrolyze in vivo and include those that break down readily in the human body to leave the parent compound or a salt thereof. Suitable ester groups include, but are not limited to, those derived from pharmaceutically acceptable aliphatic carboxylic acids, particularly alkanoic, alkenoic, cycloalkanoic and alkanedioic acids, in which each alkyl or alkenyl moiety advantageously has not more than 6 carbon atoms. Examples of particular esters include, but are not limited to, formates, acetates, propionates, butyates, acrylates and ethylsuccinates.

The term " pharmaceutically acceptable prodrugs" as used herein refers to those prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, commensurate with a reasonable risk/reward ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "prodrug" refers to compounds that are rapidly transformed in vivo to yield the parent compound of the above formulae, for example, by hydrolysis in blood. A thorough discussion is provided in T. Higuchi and V. Stella, Prodrugs as Novel delivery Systems, Vol. 14 of the A.C.S. Symposium Series and in Edward B. Roche, ed., Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated by reference herein.

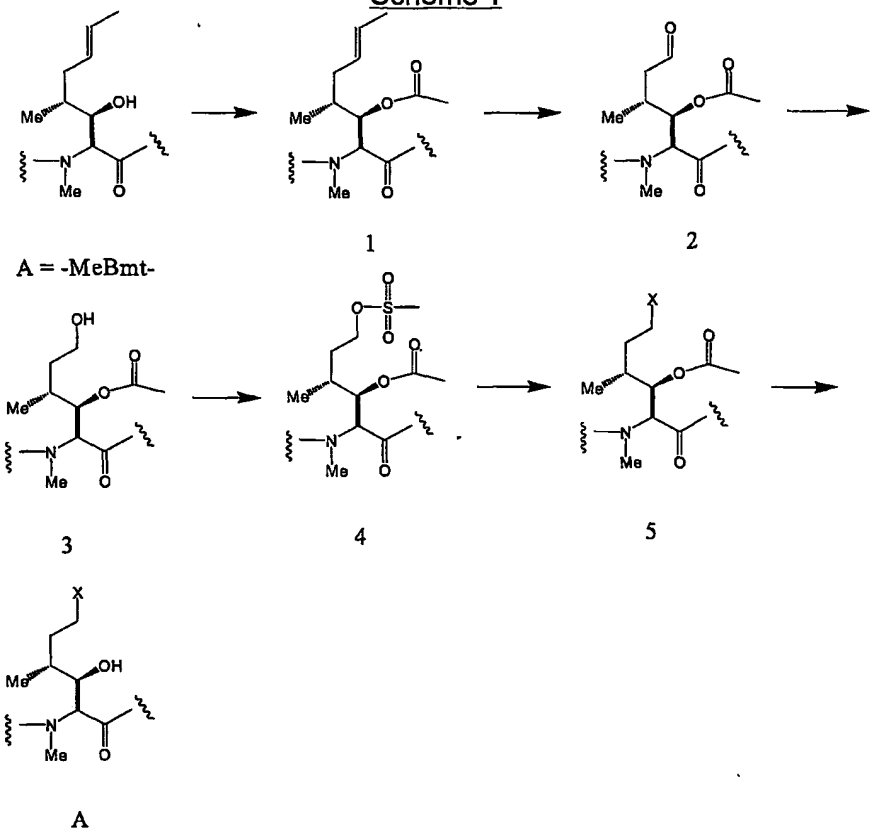
Abbreviations

Sar:	Sarcosin
Ac:	Acetyl
MeLeu:	N-Methyl-Leucine
Val:	Valine
Ala:	Alanine
MeVal:	N-Methyl Valine
Et:	Ethyl
Ph:	Phenyl
MeBmt:	N-Methyl-butenyl-threonine

Synthetic Methods

The compounds and processes of the present invention will be better understood in the following synthetic scheme which illustrates the methods by which the compounds of the present invention may be prepared. The groups B and U in Formula I are as defined above. A is –MeBmt- in the starting material as illustrated in Scheme 1:

Scheme 1



The process for the invention for the preparation of the compounds of Formula I comprises reacting cyclosporin A, a commercially available fermentation product wherein A = -MeBmt- with acetic anhydride, optionally in the presence of pyridine or dimethylaminopyridine, in dichloromethane to give acetylated cyclosporin A intermediate **1** (see Eberle, M. K., Nuninger, F. J. Org. Chem. 1992, 57, 2689-2691). Ozonolysis of intermediate **1** carried out at –78°C in dichloromethane, followed by quenching with dimethylsulfide gives the aldehyde **2** (see Park, S. B., Meier, G. P. Tetrahedron Lett. 1989, 30, 4215-4218). Reduction of the aldehyde Intermediate **2** at 0°C with sodium borohydride gives the alcohol **3** (see Toshima, U., Tatsuta, K., Kinoshito, M. Bull. Chem. Soc. Jpn 1988, 61, 2369;

Colombo, L., Di Giacomo, M. Tetrahedron Lett. 1999, 40, 1977), which is reacted with methanesulfonyl chloride and triethylamine in dichloromethane to give intermediate **4** (see Kitahara, T., Matsuoka, T., Katayama, M., Maramo, S., Mori, K. Tetrahedron Lett. 1984, 25, 4685; Ireland, R. E., Anderson, R. C., Badoud, R., Fitzsimmons, B. J. J. Am. Chem. Soc. 1983, 105, 1988). Intermediate **4** can be converted to Intermediate **5** by displacement with a nucleophile, such as, but not limited to sodium azide, sodium phenoxide, sodium thiophenoxide, sodium cyanide in dimethylformamide or tetrahydrofuran at room temperature to 60°C for 3 to 48 hours (see Effenberger, F., Stelzer, U. Angew. Chem. 1991, 103, 866; Fleming, P. R., Sharpless, K. B. J. Org. Chem. 1991, 56, 2869). Intermediate **5** is then converted to the compound of Formula I by hydrolysis with potassium carbonate in methanol (see Plattner, J. J., Gless, R. D., Rapoport, H. J. Am. Chem. Soc. 1972, 94, 8613).

Pharmaceutical Compositions

In the pharmaceutical compositions of the present invention, a compound of the invention is combined with a pharmaceutically acceptable carrier or excipient, meaning a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or Formulation auxiliary of any type. The compositions may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Pharmaceutical compositions of this invention for parenteral injection comprise pharmaceutically-acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity may be maintained, for example, by the use of coating materials such as

lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservative, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of the drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters) and poly(anhydrides). Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

The injectable formulations may be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically-acceptable excipient or carrier, such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such

as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules may be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically-acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Suspensions may contain, in addition to the active compounds, suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol

and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures thereof.

Topical administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for topical administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized powder compositions, the active ingredient in finely divided form may be used in admixture with a larger-sized pharmaceutically-acceptable inert carrier comprising particles having a size, for example, of up to 100 micrometers in diameter. Suitable inert carriers include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers.

Alternatively, the composition may be pressurized and contain a compressed gas, such as nitrogen or a liquified gas propellant. The liquified propellant medium and indeed the total composition are preferably such that the active ingredient does not dissolve therein to any substantial extent. The pressurized composition may also contain a surface-active agent, such as a liquid or solid non-ionic surface-active agent or may be a solid anionic surface-active agent. It is preferred to use the solid anionic surface-active agent in the form of a sodium salt.

A further form of topical administration is to the eye, as for the treatment of immune-mediated conditions of the eye such as autoimmune diseases, allergic or inflammatory conditions, and corneal transplants. The compound of the invention is delivered in a pharmaceutically acceptable ophthalmic vehicle, such that the compound is maintained in contact with the ocular surface for a sufficient time period to allow the compound to penetrate the corneal and internal regions of the eye, as for example the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/cilary, lens, choroid/retina and sclera. The pharmaceutically acceptable ophthalmic vehicle may, for example, be an ointment, vegetable oil or an encapsulating material.

Compositions for rectal or vaginal administration are preferably suppositories which may be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene

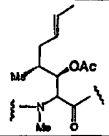
glycol or a suppository wax which are solid at room temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Compounds of the present invention may also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (l976), p. 33 *et seq.*

Examples

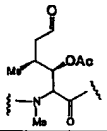
The procedures described above for preparing the compounds of the present invention will be better understood in connection with the following examples, which are intended to be illustrative only and not limiting of the scope of the invention. Various changes and modifications of the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation, those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, formulations and/or methods for the invention may be made without departing from the spirit of the invention and the scope of the appended claims.

Example 1.

Compound of Formula (I): A =  , B = –αAbu- and U = –(D)Ala-.

To a solution of cyclosporin A (20 g, 16.6 mmol) in methylene chloride (40 mL) were added pyridine (10.07 mL, 124.5 mmol), dimethylaminopyridine (2.03 g, 16.6 mmol) and acetic anhydride (7.83 mL, 83 mmol) dropwise. The reaction mixture was stirred at ambient temperature for 18 hours. Subsequently, the mixture was diluted with ethyl acetate and washed with 1N HCl, 1M NaOH and brine. The organic layer was dried over magnesium sulfate and concentrated *in vacuo* to give the title compound as a white solid (20.7 g, 100% yield). Electrospray mass spectrum (ESMS) M+H: 1244.48.

Example 2.

Compound of Formula (I): A =  , B = –αAbu- and U = –(D)Ala-.

A solution of the cyclosporin acetate-protected derivative from Example 1 (20.7 g, 16.6 mmol) in methylene chloride (40 mL) was cooled to -78°C with a dry ice/acetone bath and ozone was bubbled through the solution until the blue color persisted. Subsequently, oxygen was bubbled through the reaction mixture for 15 minutes and the reaction was quenched with dimethylsulfide (4 mL) and allowed to warm to ambient temperature overnight. The solution was then concentrated *in vacuo* to afford the title compound as a clear oil (20.5 g, 100% yield). ESMS M+H: 1232.31.

Example 3.

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = OH, R = Ac, B = –αAbu- and U = –(D)Ala-

A solution of the cyclosporin acetate-protected aldehyde derivative from Example 2 (20.5 g, 16.6 mmol) in anhydrous methanol (30 mL) was cooled to below 0°C with a water/brine bath and sodium borohydride (6.28 g, 166 mmol) was added slowly over 30 minutes. After 1 hour, the reaction was quenched with water and 1 N HCl and diluted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate and concentrated *in vacuo* to give the title compound as a white solid (16.26g, 79% yield).

ESMS M+H: 1234.37.

Example 4.

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = $OS(O)_2CH_3$, R = Ac, B = –αAbu- and U = –(D)Ala-.

To a cold (0°C) solution of the cyclosporin acetate-protected alcohol derivative from Example 3 (15.33 g, 12.42 mmol) in methylene chloride (200 ml) was added methanesulfonyl chloride (1.44 mL, 18.63 mmol) and triethylamine (5.2 mL, 37.26 mmol) and the reaction was stirred at 0°C for 3 hours and then stored at 4°C for 18 hours. Subsequently, the reaction was diluted with ethyl acetate washed with saturated sodium bicarbonate, brine and dried over magnesium sulfate. After concentration *in vacuo*, the title compound was obtained as an orange solid (14.63 g, 90% yield).

ESMS M+H: 1312.53.

Example 5.

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = N_3, R = Ac, B = –αAbu- and U = –(D)Ala-.

Sodium azide (7.2 g, 111.6 mmol) was added to a solution of the cyclosporin acetate-protected mesylate derivative from Example 4 (14.63 g, 11.16 mmol) in dimethyl formamide (30 mL) and the reaction was heated to 60°C for 18 hours. Subsequently, ethyl acetate was added and the mixture was washed with saturated sodium bicarbonate and brine. Drying over magnesium sulfate and concentration in vacuo gave the title compound as an off-white solid (13.6 g, 97% yield).

ESMS M+H: 1260.38.

Example 6.

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = N_3, R = H, B = –αAbu- and U = –(D)Ala-.

Potassium carbonate (7.45 g, 54.05 mmol) was added to a solution of the cyclosporin acetate-protected azide derivative from Example 5 (13.6 g, 10.81 mmol) in anhydrous methanol (100 mL) and the reaction was stirred at ambient temperature for 18 hours. Subsequently, ethyl acetate was added and the mixture was washed with saturated sodium bicarbonate and brine. The organic layer was

dried over magnesium sulfate and concentrated *in vacuo* to give te title compound as a yellow solid (11.57 g, 88 % yield).
ESMS M+H: 1218.16.

Example 7.

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = CN, R = Ac, B = –αAbu- and U = –(D)Ala-.

The title compound was prepared by reacting the title compound of Example 4 with sodium cyanide according to the procedures described in Example 5.
ESMS M+H: 1242.83.

Example 8.

Compound of Formula (I), (A): X = -$(CH_2)_2$-, Y = CN, R = H, B = –αAbu-, U = –(D)Ala-.

The title compound of was prepared by reacting the title compound of Example 7 with potassium carbonate in methanol according to the procedures described in Example 6.
ESMS M+H: 1200.82.

Example 9.

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = F, R = Ac, B = –αAbu- and U = –(D)Ala-.

The title compound was prepared by reacting the title compound of Example 4 with tetra-n-butyl-ammonium fluoride according to the procedures described in Example 5.
ESMS M+H: 1235.83.

Example 10.

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = F, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound was prepared by reacting the title compound of Example 9 with potassium carbonate in methanol according to the procedures described in Example 6.
ESMS M+H: 1193.82.

Example 11.

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = Cl, R = Ac, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 4 with tetra-n-butyl-ammonium chloride according to the procedures described in Example 5.

Example 12.

Compound of Formula (I), (A): X = -$(CH_2)_2$-, Y = Cl, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 11 with potassium carbonate in methanol according to the procedures described in Example 6.

Example 13.

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = Br, R = Ac, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 4 with tetra-n-butyl-ammonium bromide according to the procedures described in Example 5.

Example 14.

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = Br, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 13 with potassium carbonate in methanol according to the procedures described in Example 6.

Example 15.

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = PhSe, R = Ac, B = –αAbu- and U = –(D)Ala-.

To a solution of diphenyl diselenide (0.032 g, 0.102 mmol) in anhydrous methanol (5 mL) was added sodium borohyride (0.0074 g, 0.192 mmol) and the solution was heated to reflux for 30 minutes. The title compound of Example 4 was added (0.2 g, 0.153 mmol) and the reaction was refluxed for 3 additional hours. Subsequently, the reaction was quenched with water and 1N HCl and was diluted with ethyl acetate. The organic layer was washed with 1N NaOH, brine and dried over magnesium sulfate. Concentration *in vacuo* gave the title compound as a yellow solid (0.13g, 92% yield).
ESMS M+H: 1374.78.

Example 16.

Compound of Formula (I): (A): X and Y taken together = -CH=CH_2, R = Ac, B = –αAbu- and U = –(D)Ala-.

To a cold (0°C) solution of the title compound of example 15 (0.4 g, 0.29 mmol) in 4/1 tetrahydrofuran/water (5 ml) was added sodium periodate (0.125g, 0.58 mmol) and the reaction was allowed with warm up to ambient temperature with stirring. After 18 hours, the reaction mixture was concentrated in vacuo, diluted with sodium bicarbonate and extracted with ethyl acetate. The organic extracts were washed with brine, dried over magnesium sulfate and concentrated in vacuo. Purification by column chromatography (silica gel, 2/1 hexane/acetone) gave the title compound as a white solid (62% yield).
ESMS M+H: 1216.82.

Example 17.

Compound of Formula (I): (A): X and Y taken together = -CHO, R = Ac, B = –αAbu- and U = –(D)Ala-.

The title compound was prepared by reacting the title compound of Example 16 with ozone according to the procedure described in Example 2.
ESMS M+H: 1218.80.

Example 18.

Compound of Formula (I): (A): X and Y taken together = -CH_2OH, R = Ac, B = –αAbu- and U = –(D)Ala-.

The title compound was prepared by reducing the title compound of Example 17 with sodium borohydride according to the procedure described in Example 3.

ESMS M+H: 1220.82.

Example 19.

Compound of Formula (I): (A): X and Y taken together = $-CH_2OS(O)_2CH_3$, R = Ac, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 18 with methanesulfonyl chloride and triethylamine according to the procedure described in Example 4.

Example 20.

Formula (I): (A): X and Y taken together = $-CH_2N_3$, R = Ac, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 19 with sodium azide according to the procedure described in Example 5.

Example 21.

Compound of Formula (I): (A): X and Y taken together = $-CH_2N_3$, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 20 with potassium carbonate in methanol according to the procedure described in Example 6.

Example 22.

Compound of Formula (I): (A): X-Y = $-CH_2CN$, R = Ac, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 19 with potassium cyanide according to the procedure described in Example 5.

Example 23.

Compound of Formula (I): (A): X and Y taken together = $-CH_2CN$, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 22 with potassium carbonate in methanol according to the procedure described in Example 6.

Example 24.

Compound of Formula (I): (A): X and Y taken together = $-CH_2F$, R = Ac, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 19 with tetra-n-butylammonium fluoride according to the procedure described in Example 5.

Example 25.

Compound of Formula (I): (A): X and Y taken together = $-CH_2F$, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 24 with potassium carbonate in methanol according to the procedure described in Example 6.

Example 26.

Compound of Formula (I): (A): X and Y taken together = $-CH=CH_2$, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound was prepared by reacting the title compound of Example 15 with potassium carbonate in methanol.

ESMS M+H: 1174.81.

Example 27.

Compound of Formula (I): (A): X and Y taken together = $-CH_2-CH_3$, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared by reacting the title compound of Example 26 with hydrogen in the presence of palladium on carbon in methanol.

Example 28.

Compound of Formula (I): (A): X and Y taken together = CH_2-CH=CH-$(CH_2)_2$-OAc, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound was prepared by reacting cyclosporine A (0.3 g, 0.25 mmol) 4-butenyl acetic acid ester (0.063 ml, 0.5 mmol) and 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylinene(tricyclohexylphosphine)dichloro ruthenium(II) benzylidene (0.042 g, 0.05 mmol) in toluene at 80°C for 18 hours. After concentation in vacuo, flash chromatography (silica gel, 2/1 hexane/acetone) afforded the title compound as an off-white solid (0.09 g, 29 % yield).
ESMS M+H: 1274.86.

Example 29.

Compound of Formula (I): (A): X and Y taken together = CH_2-CH=CH-$(CH_2)_3$-OAc, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound was prepared by reacting cyclosporine A with 4-pentenyl acetic acid ester and 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylinene(tricyclohexylphosphine)dichloro ruthenium(II) according to the procedures described in Example 28.
ESMS M+H: 1288.88.

Example 30.

Compound of Formula (I): (A): X and Y taken together = CH_2-CH=CH-$(CH_2)_4$-OAc, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound was prepared by reacting cyclosporine A with 4-hexenyl acetic acid ester and 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylinene(tricyclohexylphosphine)dichloro ruthenium(II) according to the procdures described in Example 28.
ESMS M+H: 1302.89.

Example 31.

Compound of Formula (I): (A): X and Y taken together = CH_2-CH=CH-$(CH_2)_3$-Br, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound was prepared by reacting cyclosporine A with 1-bromo-5-pentene and 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-

ylinene(tricyclohexylphosphine)dichloro ruthenium(II) according to the procedures described in Example 28.

ESMS M+H: 1308.78.

Example 32.

Compound of Formula (I): (A): X and Y taken together = - $(CH_2)_5$-OAc, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound is prepared from the title compound of Example 28 and hydrogen in the presence of palladium on carbon.

Example 33.

Compound of Formula (I): (A): X and Y taken together = -$(CH_2)_5$-N_3, R = H, B = –αAbu- and U = –(D)Ala-.

The title compound of is prepared from the title compound of Example 32 and sodium azide in the presence of a catalytic amount of potassium iodide.

Example 34.

Compound of Formula (I): (A): X and Y taken together = CH_2-CH=CH-$(CH_2)_3$-CN, R = H, B = –αAbu-, U = –(D)Ala-

The title compound is prepared by reacting the title compound of Example 28 with potassium cyanide in the presence of a catalytic amount of potassium iodide.

The cyclosporins of the present invention have potent immunosuppressive anti-inflammatory activity. In particular they inhibit antigen-induced inflammatory cell infiltration, for example, into the airways. *In vivo* this activity is apparent following topical administration, e.g., via the pulmonary route.

Anti-inflammatory properties of the cyclosporins of the invention may be demonstrated in standard test models *in vitro* and *in vivo*, e.g., as follows.

Example 35: Calcineurin Inhibition Assay

The immunosuppressive activity of cyclosporin is mediated through inhibition of the phosphatase activity of the enzyme calcineurin by a cyclophilin-cyclosporin complex. Thus, calcineurin inhibition is widely used as an *in vitro* measure of the activity of cyclosporin analogs.

Compounds were tested in an assay based on the Biomol Green Calcineurin Assay Kit supplied by Biomol (Plymouth Meeting, PA), supplemented with cyclophilin A for enzyme inhibition. The activity of the recombinant human calcineurin was determined by release of phosphate from a phosphopeptide representing a fragment of camp-dependent protein kinase. Phosphate release was determined using the colorimetric detection reagent Biomol Green.

Compounds in DMSO (2.4 μl) were added to a 96-well microplate and mixed with 50 μl assay buffer (50 mM Tris, pH 7.5, 0.1 M sodium chloride, 6 mM magnesium chloride, 0.5 mM dithiothreitol, 0.025% NP-40, 0.5 mM calcium chloride, 0.25 μM calmodulin) containing 5 μM cyclophilin and 20 units of calcineurin. After warming to 37 °C for 15 min, the enzymatic reaction was initiated by addition of phosphopeptide (7.5 μl) to give a final concentration of 94 μM. Phosphate release after 60 min at 37 °C was determined by addition of Biomol Green (100 μl) and measurement of the absorbance at 620 nm after 15 min at room temperature.

IC_{50} values were calculated from determinations of enzyme activity at inhibitor concentrations ranging from 20 to 0.006 μM.

Example 36: Immunosuppressive Activity and Applications

Murine Mixed Lymphocyte Reaction

Approximately $0.5x10^6$ lymphocytes from the spleen of female (8-10 weeks) Balb/c mice are incubated for 5 days in 0.2 ml cell growth medium with ca. 0.5 x 10^6 lymphocytes from the spleen of female (8-10 weeks) CBA mice. Test substance is added to the medium at various concentrations. Activity is assessed by ability to suppress proliferation-associated DNA synthesis as determined by incorporation of radiolabelled thymidine.

Mishell-Dutton Test

Approximately 10^7 lymphocytes from the spleen of OFI, female mice are co-cultured with ca. $3x10^7$ sheep erythrocytes for 3 days. Test substance is added to the incubation medium in varying concentrations. Lymphocytes are harvested and plated onto agar with fresh sheep erythrocytes as antigen. Sensitized lymphocytes secrete antibody that coats the erythrocytes, which lyse to form a plaque in the presence of complement. Activity is assessed by reduction in the number of plaque forming, i.e., antibody product, cells.

Influence on Allergen-Induced Pulmonary Eosinophilia (*in vitro*)

Male Himalayan spotted guinea pigs (300 g, BRL) are sensitized to ovalbumin (OA) by i.p. injection of 1 ml of a suspension of OA (10 µg/ml) with $Al(OH)_3$ (100 mg) and B-pertussis vaccine (0.25 ml) in saline (0.9% w/v). For oral studies the procedure is repeated 1x after 2 weeks and the animals are used one week later. For inhalation studies the procedure is repeated 2x at 3-week intervals and the animals are used one week after the last injection.

Challenge is affected employing a saline solution of OA, nebulized for discharge into an exposure chamber. Test animals are exposed to OA by nose-only inhalation for 60 minutes. For inhalation studies, OA solution is used at a concentration of 0.01%.

Test substance is administered by inhalation and/or orally. For oral studies, test substance is administered p.o. in olive oil 1x daily for 3 days or in powder form in methylcellulose once prior to OA challenge. On day 3, test animals receive test substance 1.5 hours prior to and 6 hours after OA challenge. For inhalation studies, test substance is micronised for delivery to test animals restrained within a flow-past, nose-only inhalation chamber. Administration by inhalation is effected 15 minutes prior to OA challenge.

Efficacy of administered test substance is determined by bronchoalveolar lavage (BAL) and cell counting. For this purpose animals are sacrificed with Na pento-barbitone (100 mg/kg i.p.) and the trachea is exposed and cannulated. 5 successive 10 ml aliqots of Ca^2 + and Mg^2 + free Hank's balanced salt solution (HBSS), containing bovine serum albumin (BSA, 0.3%), EDTA (10mM) and HEPES (10 mM) is then introduced into the lung and immediately aspirated by gentle compression of the lung tissue. Total cell counts in pooled eluates are determined using an automatic cell counter. Lavage fluid is centrifuged at 200g for 10 minutes and the cell pellet resuspended in 1 ml of supplemented HBSS. 10 µl of this cell suspension is added to 190 µl of Turk's solution (1:20) dilution). Differential cell counts are made from smears stained by Diff-Quick. Cells are identified and counted under oil immersion (x1,000). A minimum of 500 cells per smear are counted and the total population of each cell type is calculated.

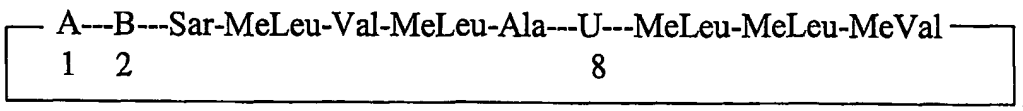
Although the invention has been described with respect to various preferred embodiments, it is not intended to be limited thereto, but rather those skilled in the

art will recognize that variations and modifications may be made therein which are within the spirit of the invention and the scope of the appended claims.

5

WHAT IS CLAIMED IS:

1. A compound of Formula (I)

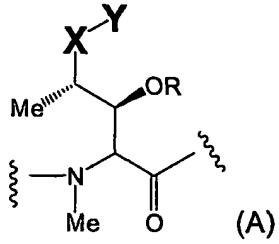


(1)

or a pharmaceutically acceptable salt, ester, or prodrug thereof:

wherein:

A is



(A)

X is selected from the group consisting of $-(CH_2)_n-$ and $-CH_2-CH=CH-(CH_2)_m-$, where n is an integer of from 1 to 8 and m is an integer from 2 to 5,

Y is selected from the group consisting of OH, OAc, halogen, N_3, CN and $OS(O)_2R_{10}$, where R_{10} is selected from the group consisting of F, CH_3, CF_3, Ph, and MePh;

or, alternatively, X and Y taken together are selected from the group consisting of $-CH=CH_2$, –CHO and $-CH_2CH_3$;

R is selected from the group consisting of hydrogen and a hydroxyl protecting group;

B is selected from the group consisting of –αAbu-, -Val-, -Thr- and –Nva-; and

U is selected from the group consisting of –(D)Ala-, -(D)Ser-, –[O-(2-hydroxyethyl)(D)Ser]-; -[O-acyl(D)Ser]- and –[O-(2-acyloxyethyl)(D)Ser]-.

2. A compound according to claim 1 which is selected from the group consisting of:

Compound of Formula (I): (A), X = $-(CH_2)_2-$, Y = OH, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = $OS(O)_2CH_3$, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = N_3, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = N_3, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = CN, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = CN, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = F, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = F, R = H, B = –αAbu- and U = –(D)Ala-,

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = Cl, R = Ac, B = –αAbu- and U = –(D)Ala-,

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = Cl, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X = -$(CH_2)_2$-, Y = Br, R = H, B = –αAbu- and U = –(D)Ala-,

Compound of Formula (I): (A): X andY taken together = -CH=CH_2, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X andY taken together = -CH=CH_2, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CH_2CH_3, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CHO, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CHO, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = -CH_2OH, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = $-CH_2OS(O)_2CH_3$, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = $-CH_2N_3$, R = Ac, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = $-CH_2N_3$, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = $-CH_2CN$, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = $-CH_2F$, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = $-CH_2Cl$, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = $-CH=CH-(CH_2)_3-OAc$, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = $CH_2-CH=CH-(CH_2)_3N_3$, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = $CH_2-CH=CH-(CH_2)_2-OAc$, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = $CH_2-CH=CH-(CH_2)_4-OAc$, R = H, B = –αAbu- and U = –(D)Ala-;

Compound of Formula (I): (A): X and Y taken together = $CH_2-CH=CH-(CH_2)_3-Br$, R = H, B = –αAbu- and U = –(D)Ala-; and

Compound of Formula (I): (A): X and Y taken together = $CH_2-CH=CH-(CH_2)_3-CN$, R = H, B = –αAbu- and U = –(D)Ala-.

3. A pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) in claim 1, or a pharmaceutically acceptable salt, ester or prodrug thereof, in combination with a pharmaceutically acceptable carrier or excipient.

4. A method of treating organ transplantation rejection in a subject, which comprises administering to said subject a therapeutically effective amount of the pharmaceutical composition of claim 3.

5. A method of treating an immune disorder in a subject, which comprises administering to said subject a therapeutically effective amount of the pharmaceutical composition of claim 3.

6. The method of treating an immune disorder in a subject as defined in claim 5, wherein said immune disorder is selected from the group consisting of psoriasis and ecxema.

7. The method of treating an immune disorder in a subject as defined in claim 6, wherein said administering is topical.

8. The method of treating an immune disorder in a subject as defined in claim 5, wherein said immune disorder is selected from the group consisting of: rheumatoid arthritis, inflammatory bowel disease, psoriasis, asthma, allergic rhinitis and chronic obstructive pulmonary disease.

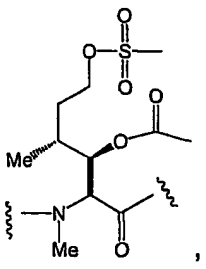
9. A method of treating inflammatory or obstructive airways disease in a subject in need of said treatment, comprising topically administering to said subject a therapeutically effective amount of the pharmaceutical composition as defined in claim 3.

10. The method of claim 9, wherein said topically administering is by inhalation.

11. The method of claim 9, wherein said airways disease is selected from the group consisting of asthma, allergic rhinitis, bronchitis, COPD including emphesema, chronic bronchitis and cystic fibrosis.

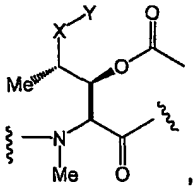
12. A process for preparing compounds of Formula (I) as defined in claim 1, the process comprising:

a. reacting a compound of Formula (I), wherein A is



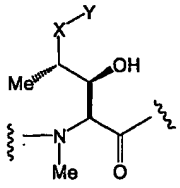
and B and U are as defined in claim 1

with a nucleophile in dimethylformamide or tetrahydrofuran at room temperature to 60°C for 3 to 48 hours to prepare a compound of Formula (I), wherein A is



and X, Y, B, and U are as defined in claim 1; and

b. hydrolyzing the compound of step a with potassium carbonate in methanol to yield a compound of Formula (I),

wherein A is  and X, Y, B and U are as defined in claim 1.

13. The process of claim 10, wherein the nucleophile is selected from the group consisting of sodium azide and sodium cyanide.